Use of selected scavengers for the determination of NF-TiO2 reactive oxygen species during the degradation of microcystin-LR under visible light irradiation

Pelaez, M., Falaras, P., Likodimos, V., O'Shea, K., de la Cruz, A. A., Dunlop, PSM., Byrne, JA., & Dionysiou, D. D. (2016). Use of selected scavengers for the determination of NF-TiO2 reactive oxygen species during the degradation of microcystin-LR under visible light irradiation. *Journal of Molecular Catalysis A: Chemical*, *425*, 183-189. https://doi.org/10.1016/j.molcata.2016.09.035

Link to publication record in Ulster University Research Portal

Published in:
Journal of Molecular Catalysis A: Chemical

Publication Status:
Published (in print/issue): 30/09/2016

DOI:
10.1016/j.molcata.2016.09.035

Document Version
Publisher's PDF, also known as Version of record

General rights
Copyright for the publications made accessible via Ulster University's Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Ulster University's institutional repository that provides access to Ulster's research outputs. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact pure-support@ulster.ac.uk.

Download date: 05/05/2023

Journal of Molecular Catalysis A: Chemical 425 (2016) 183–189

Contents lists available at ScienceDirect

Journal of Molecular Catalysis A: Chemical

journal homepage: www.elsevier.com/locate/molcata

Editor's choice paper

Use of selected scavengers for the determination of NF-TiO_2 reactive oxygen species during the degradation of microcystin-LR under visible light irradiation

Miguel Pelaez[a], Polycarpos Falaras[b], Vlassis Likodimos[c], Kevin O'Shea[d], Armah A. de la Cruz[e], Patrick S.M. Dunlop[f], J. Anthony Byrne[f], Dionysios D. Dionysiou[a,*]

[a] Environmental Engineering and Science Program, University of Cincinnati, Cincinnati, OH, 45221-0012, USA
[b] Institute of Nanoscience and Nanotechnology, NCSR Demokritos, 15310, Aghia Paraskevi, Attikis, Athens, Greece
[c] Section of Solid State Physics, Department of Physics, National and Kapodistrian University of Athens, Panepistimioupoli, 157 84, Athens, Greece
[d] Department of Chemistry and Biochemistry, Florida International University, University Park, Miami, FL, 3319, USA
[e] Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268, USA
[f] Nanotechnology and Integrated BioEngineering Centre, School of Engineering, Ulster University, Northern Ireland, BT37 0QB, United Kingdom

ARTICLE INFO

Article history:
Received 30 August 2016
Received in revised form
27 September 2016
Accepted 29 September 2016
Available online 30 September 2016

Keywords:
NF-TiO_2
Reactive oxygen species
Hydroxyl radical
Superoxide radical anion
Singlet oxygen
Visible light
Microcystin-LR

ABSTRACT

Although UV-induced TiO_2 photocatalysis involves the generation of several reactive oxygen species (ROS), the formation of hydroxyl radicals is generally associated with the degradation of persistent organic contaminants in water. In this study, a variety of radical scavengers were employed to discriminate the roles of different ROS during visible light-activated (VLA) photocatalysis using nitrogen and fluorine doped TiO_2 (NF-TiO_2) in the degradation of the hepatotoxin, microcystin-LR (MC-LR) in water. The addition of hydroxyl radical scavengers, methanol and *tert*-butyl alcohol to the reaction mixture resulted in negligible inhibition of NF-TiO_2 photocatalytic degradation of MC-LR at pH 3.0 and only partial inhibition at pH 5.7 under visible light. While hydroxyl radicals (•OH) generally play the primary role in UV-TiO_2 photocatalysis, the minimal influence of MeOH and t-BuOH on the degradation process under these experimental conditions indicates that •OH are not crucial in VLA NF-TiO_2 photocatalysis. However, strong inhibition was observed in VLA NF-TiO_2 photocatalytic degradation of MC-LR in the presence of superoxide dismutase, benzoquinone and catalase at pH 3.0 and 5.7 indicating that $O_2^{\bullet-}$ and H_2O_2 play critical roles in the degradation process. Similar degradation rates were observed in the presence of deuterium oxide, which enhances singlet oxygen mediated processes further suggesting singlet oxygen is not a key species in the degradation of MC-LR. Formic acid and cupric nitrate were added to probe the roles of the valence band holes and conduction band electrons, respectively. Under UV–vis light irradiation, almost complete inhibition of MC-LR removal is observed with NF-TiO_2 in the presence of •OH scavengers at pH 5.7. These results demonstrate that the solution pH plays a major role in the formation and reactivity of ROS during VLA NF-TiO_2 photocatalysis. The adsorption strength of scavengers and MC-LR onto NF-TiO_2 as well as the speciation of ROS as a function of pH needs to be carefully considered since they also play a major role in the efficiency of the process. These results indicate that the reduction of molecular oxygen by photo-generated electrons rather than hydroxyl radicals produced by oxidative reactions of photo-generated holes is the key factor in the VLA NF-TiO_2 photocatalytic degradation of MC-LR.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The primary electronic processes in TiO_2 photocatalysis under UV irradiation involve the absorption of a photon with energy equal or greater to its band gap (3.2 eV for anatase and brookite TiO_2 and 3.0 eV for rutile TiO_2) that excites an electron to the conduction band (e^-_{CB}) generating a hole in the valence band (h^+_{VB}) [1–4]. These charge carriers can migrate to the catalyst surface and initiate redox reactions, where the h^+_{VB} can oxidize OH^- or water adsorbed at the surface to produce reactive oxygen species (ROS), mainly hydroxyl radicals (•OH), while e^-_{CB} can be scavenged by adsorbed molecular oxygen leading to the formation of

* Corresponding author.
E-mail address: dionysios.d.dionysiou@uc.edu (D.D. Dionysiou).

http://dx.doi.org/10.1016/j.molcata.2016.09.035
1381-1169/© 2016 Elsevier B.V. All rights reserved.

superoxide radical anions ($O_2^{\bullet-}$). $O_2^{\bullet-}$ may further react to form singlet oxygen and hydrogen peroxide, the latter producing •OH as well [1]. Interfacial charge transfer from the photogenerated electron/holes to acceptor/donor species at the surface competes with charge recombination at surface trapping sites or in the bulk of TiO_2. Recombination of the electron/hole pair results in loss of adsorbed photonic energy with release of heat by non-radiative decay processes. The generation of ROS by TiO_2 photocatalysis upon illumination by UV light along with its environmentally benign properties (i.e. non-toxicity, absence of dissolution in water, photostability) and relatively low cost, render TiO_2 photocatalysis a more favorable process for the remediation of recalcitrant organic pollutants in water and air as well as for killing pathogenic microorganisms [2–4]. Despite the marked progress in TiO_2 photocatalytic materials, their practical application is challenged by two inherent limitations of TiO_2: 1) the low quantum yield i.e., the ratio of reactant molecules destroyed or the product molecules formed during the photocatalytic reaction to the quantity of absorbed photons at a given wavelength [1], which is primarily determined by the recombination of photogenerated charge carriers, and 2) the relatively poor solar light harvesting that is limited by the wide band gap of TiO_2 in the UVA spectral range. Both factors undermine the photocatalytic efficiency of TiO_2 and its enormous potential as an essential "green" technology for the destruction of noxious organic compounds in water and air under solar excitation. The modification and further development of TiO_2-based photocatalytic technology towards visible light activation will provide an opportunity to develop nanocatalysts that can absorb both UV (280–400 nm) and visible (400–700 nm) radiation to enhance the overall process efficiency and employ solar light as a renewable source of energy. Visible light activation of TiO_2 employing various approaches such as photosensitization with dyes, modification with metal and non-metal atoms and coupling with narrow band gap semiconductors are areas actively being pursued [5–9]. Nevertheless, the photocatalytic degradation mechanisms associated with visible light-activated (VLA) TiO_2 photocatalysis are still ambiguous and the formation as well as the role of different ROS remains unclear.

In dye-sensitized TiO_2 photocatalysis, the dye absorbs visible light instead of TiO_2 and becomes photo-excited [6]. The electrons from the excited dye molecule are subsequently transferred into the conduction band of TiO_2 and react with molecular oxygen to produce ROS. Recent work by Diaz-Uribe et al., suggests the formation of $O_2^{\bullet-}$ with tetra (4-carboxyphenyl) porphyrin sensitized TiO_2 but no singlet oxygen was detected using electron paramagnetic resonance (EPR); even though the possibility was not excluded completely [10]. Goldstein et al., concluded that oxidation of methanol was governed by the generation of surface holes followed by organic radical-based attack in carbon doped TiO_2 photocatalysis under VLA [11]. Based on EPR studies using spin traps, Fu et al., provided evidence that •OH and $O_2^{\bullet-}$ were responsible for the photodegradation of 4-CP by VLA activated nitrogen-doped TiO_2 (N-TiO_2) nanoparticles [12]. For nitrogen and sulfur-doped TiO_2 nanoparticles, EPR spin trapping studies suggest $O_2^{\bullet-}$ and singlet oxygen were formed upon visible light illumination and were responsible for the inactivation of *Escherichia coli* in water [13]. A chemiluminescence technique quantified an increase of singlet oxygen production when using a composite TiO_2/N-TiO_2 photocatalyst under visible light irradiation for NO_x decomposition compared to N-TiO_2 only. As for TiO_2, the characteristic 1268 nm emission of singlet oxygen was not detected upon visible light irradiation [14]. Recent developments and insights in this field highlight the need to further develop and understand the mechanisms underlying the reactivity of VLA photo-catalysts for environmental remediation [15,16].

Table 1
List of compounds selected as scavengers for •OH, $O_2^{\bullet-}$, $^1O_2$, H_2O_2, e^-, and h^+.

Compound	Scavenger type
Formic acid	h^+ scavenger
Cupric Nitrate	e^- scavenger
Methanol	•OH
Tertbutanol	•OH
D_2O[a]	$^1O_2$
1,4-benzoquinone	$O_2^{\bullet-}$
Superoxide dismutase	$O_2^{\bullet-}$
Catalase	H_2O_2

[a] Lifetime of singlet oxygen is 55 μsec in D_2O compared to 4.2 μsec in H_2O [34]. It is not an actual scavenger (see Section 2.1).

Therefore, the main objective of this study was to elucidate the formation of ROS with a visible light-activated TiO_2 photocatalyst using selected chemical probe scavengers that are specific for •OH, $O_2^{\bullet-}$, singlet oxygen and the photogenerated electron-hole pairs. NF-TiO_2 has been successfully synthesized, characterized and evaluated for its photocatalytic activity under visible light [17,18]. The use of scavengers will contribute to a better understanding of the photocatalytic mechanism of NF-TiO_2 towards the degradation of the cyanobacterial toxin microcystin-LR (MC-LR). MC-LR is a highly toxic and persistent cyanotoxin commonly detected in cyanobacterial harmful algae blooms in surface water [19,20]. The presence of these toxins in recreational water bodies and drinking water sources is a serious concern to the environmental and health authorities because of the significant health risk to humans and the environment.

2. Materials and methods

2.1. Chemicals and NF-TiO_2 synthesis

Methanol (MeOH, Tedia) and *tert*-butyl alcohol (TBA, Fisher) were used as hydroxyl radical scavengers. D_2O is used to prolong the lifetime of singlet oxygen. For the inhibition of superoxide radical anion, 1,4-benzoquinone (98%, Sigma Aldrich) and superoxide dismutase (SOD, Sigma Aldrich) from *Escherichia coli* (manganese-containing enzyme, lyophilized, activity ≥1000 units/mg protein) were employed. The electron and hole pair were scavenged with cupric nitrate (Fisher) and formic acid (88% Fisher), respectively. The nitrate salt of Cu^{2+} was used since adsorption of the nitrate anions onto TiO_2's surface is weak. Catalase from bovine liver was obtained from Sigma Aldrich and used as H_2O_2 inhibitor. L-histidine (98%, Acros Organics) was used during UV irradiation to scavenge singlet oxygen and hydroxyl radicals. Nitric acid was used to adjust the pH at 3.0 in experiments dealing with acidic pH. Finally, MC-LR standard (dry solid, 500 μg) was obtained from Calbiochem. The nanotechnological approach for the synthesis of NF-TiO_2 consisted of a nonionic fluorosurfactant as pore template and fluorine dopant and ethylenediamine as nitrogen dopant, as previously described in detail in [17]. The resulting nanoparticles exhibited high BET surface area (141 m^2/g), high porosity (49%), small crystallite size (8.7 nm) with nitrogen and fluorine content of 1.5% and 4.9%, respectively [17].

2.2. Scavenger experiments

A borosilicate petri dish was used as reactor and MilliQ-grade water was adjusted to the desired pH and a specific scavenger was added into solution to obtain an initial concentration of 5000 μM except for SOD and catalase, where 2 μM and 10 μM were employed (see Table 1 for selected scavengers). The solution was then spiked with an aliquot of MC-LR standard to reach an initial concentration of 500 ± 10 μg L^{-1}, which is equivalent to

0.5 ± 0.1 μM. Separately, an aqueous NF-TiO_2 suspension was prepared, sonicated and transferred to the reactor to achieve an initial concentration of 0.5 g L^{-1} and a final volume of 10 ml. The reactor was sealed with parafilm and mixed continuously during the photocatalytic reaction under visible light irradiation. To obtain visible light irradiation only, a UV block filter (UV420, Opticology) was mounted below two 15 W fluorescent lamps (Cole-Parmer) to eliminate photons below 420 nm. Samples were taken at specific periods of time and the samples were quenched with methanol, filtered (L815, Whatman) to remove the suspended nanoparticles, transferred to 0.2 ml glass inserts and placed in sample vials. MC-LR samples were analyzed by liquid chromatography (LC, Agilent Series 1100). The analytical conditions were similar to those reported by Antoniou et al., [20] but the column employed was a C_{18} Discovery (Supelco) column (150×2.1 mm, 5.0 μm particle size). The flow rate was 0.2 ml/min, the injection volume was 20 μl and the column temperature was 40 °C.

3. Results and discussion

3.1. •OH scavengers

To probe the role of hydroxyl radical in the photocatalytic degradation of MC-LR by VLA NF-TiO_2, a number of experiments were conducted in the absence and presence of methanol or TBA at pH 3.0 and 5.7 (see Fig. 1a and b). Methanol and TBA react rapidly with hydroxyl radical and have been used extensively as •OH scavengers to determine the presence and role of hydroxyl radicals in TiO_2 photocatalysis under UV light. The addition of methanol and TBA dramatically reduces the degradation rate of the targeted contaminants by conventional UV TiO_2 photocatalysis indicating that •OH mediated oxidation processes are the predominant pathways leading to degradation under UV irradiation [21]. The solution pH influences the overall charge on the catalyst surface and the speciation of the ionizable functional groups present in MC-LR. The surface charge and specific functional groups can lead to repulsive or attractive interaction between MC-LR and the catalyst surface. Under modest acidic conditions the catalyst surface will be positive (NF-TiO_2 point of zero charge is 6.4 [17]), while the carboxylate group will be negative down to approximately pH 4 and thus a strong electrostatic attraction is expected to enhance adsorption of MC-LR in this pH range. The hydrophobic character of MC-LR also increases with solution acidity, which may also contribute to increased adsorption and degradation under acidic conditions. The addition of 5 mM of methanol or TBA did not lead to significant inhibition of the degradation of MC-LR by VLA NF-TiO_2 photocatalysis at pH 3.0, as illustrated in Fig. 1a. The absence of inhibition in the presence of these scavengers suggests the minor role of •OH in the degradation of MC-LR under these experimental conditions. At higher pH of 5.7, partial inhibition was observed for the degradation of MC-LR in the presence of methanol and TBA. The adsorption of MC-LR onto the photocatalyst decreased as pH increased from 3.0 to 5.7 [18]. As mentioned before, MC-LR is quite hydrophobic at pH 3.0, which can enhance adsorption, while methanol and TBA may not compete with the ROS formed. With increasing pH, the adsorption of MeOH and TBA may also increase leading to lower adsorption levels for MC-LR and thus effectively reduce its degradation. Thus, methanol or TBA can adsorb to a higher extent on the surface of NF-TiO_2. Given the high relative concentrations of scavengers and decreased adsorption of MC-LR with increasing pH these conditions may lead to the blocking of the photocatalytically active sites of NF-TiO_2 and/or partial suppression of the formation of the ROS, resulting in slower MC-LR degradation. The pKa of superoxide radical anion is 4.6, which lies between 3.0 and 5.7 and this can have a pronounced effect on the subsequent chemistry of the radical. For instance, negatively charged superoxide radical anion may remain at a positively charged surface and thus inhibit disproportionation, while facilitating other pathways with singlet oxygen production.

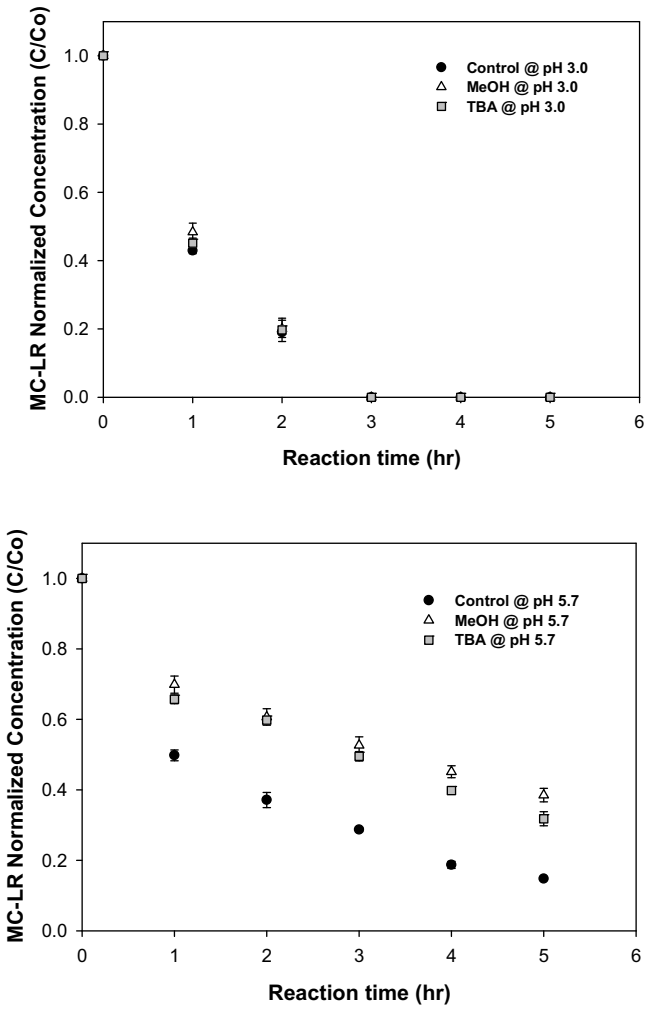


Fig. 1. Photocatalytic degradation of MC-LR with NF-TiO_2 in the absence and presence of methanol and *tert*-butyl alcohol as •OH scavengers under visible light at a) pH 3.0 b) pH 5.7.

It is well known that when unmodified anatase TiO_2 is subject to UV irradiation, the primary formation route of •OH is based on the oxidation of OH^- or water at the TiO_2 surface through the highly oxidizing and delocalized h^+_{VB} that migrate to the catalyst surface [1,3,4]. Also, the formation of H_2O_2 (that occurs upon the reduction of molecular oxygen acting as an electron acceptor) can also generate •OH via homolytic scission in a secondary reaction pathway [1] although it is highly influenced by pH. In the case of non-metal doped TiO_2, in particular nitrogen-doped TiO_2, it has been proposed that the introduction of single N hetero-atoms in the TiO_2 lattice (substitutional or interstitial) induces localized electronic states within the titania band gap and selectively promotes electrons from these states to the conduction band upon visible light irradiation [22]. Under this condition, the photogenerated holes in the localized intra-gap energy states may not have a positive enough electrochemical reduction potential to generate hydroxyl radicals at the catalyst surface from oxidation of absorbed water or hydroxyl groups. However, the formation of •OH radicals through the reaction of photoinduced e^- and/or $O_2^{\bullet -}$ with H_2O_2 ($H_2O_2 + H^+ + e_{CB} \rightarrow {}^\bullet OH + H_2O$; $H_2O_2 + O_2^{\bullet -} \rightarrow {}^\bullet OH + OH^- + O_2$) as secondary reaction pathways should not be discarded (see Section 3.2 for $O_2^{\bullet -}$ and H_2O_2 forma-

tion). Under visible light irradiation, the direct photolysis of H_2O_2 to produce •OH can be neglected due to the small molar absorption coefficient (<1 M^{-1} cm^{-1} above 300 nm) [23]. Nevertheless, the formation of •OH from H_2O_2 (with e^-_{CB}) is expected to be appreciably slower than that through surface holes at least under UV excitation according to the corresponding reduction potentials (H_2O_2/•OH is about 0.87 V (vs NHE) [24] and H_2O/•OH is about 2.27 V (vs NHE) [13] at pH 7.0 because of the multiple steps and competing pathways required to produce •OH from $O_2^{\bullet-}$.

Wang et al., [21] reported that the degradation rate of methyl orange by visible light photocatalysis using nitrogen-doped titania nanobelts was not inhibited in the presence of methanol. The authors suggested that, the generation of electrons and $O_2^{\bullet-}$ was the rate limiting step for visible light N-doped TiO_2 photocatalysis. Mrowetz et al., [25] showed that N-doped TiO_2 photocatalysis under visible light irradiation unlike UV-mediated TiO_2 photocatalysis is not capable of oxidizing $HCOO^-$. The lack of formate oxidation suggests that free or adsorbed •OH radicals are not produced under the experimental conditions tested. While water splitting with carbon doped TiO_2 under visible light has been reported, the results are subject to controversy based on photoelectrochemical studies [26].

The second order rate constants of TBA and MeOH with •OH have been reported to be 4.8×10^8 and 8.3×10^8 M^{-1} s^{-1}, respectively [27]. Literature values for the second order rate constant for MC-LR and •OH range from 1.0–2.3 (± 0.1) $\times 10^{10}$ M^{-1} s^{-1} [28]. If hydroxyl radicals were the primary species leading to the degradation of MC-LR during VLA NF-TiO_2 photocatalysis, addition of TBA or methanol should inhibit the degradation. The minimal level of inhibition observed upon addition of hydroxyl radical indicates that the hydroxyl-radical-based oxidation pathway does not play a significant role in the degradation of MC-LR with NF-TiO_2 under visible light irradiation (at the conditions tested) although the •OH formation can undergo multistep process through reduction of molecular oxygen to $O_2^{\bullet-}$, disproportionation to H_2O_2 and subsequent reduction to •OH.

3.2. $O_2^{\bullet-}$ scavengers

SOD and 1,4-benzoquinone were used as scavengers to probe the formation and role of $O_2^{\bullet-}$ during VLA NF-TiO_2 photocatalysis. The inhibition of MC-LR degradation increases with increasing SOD from 2 to 10 μM at pH 3.0, as illustrated in Fig. 2a. Significant inhibition of MC-LR was also observed in the presence of 1,4-benzoquinone. These results suggest that $O_2^{\bullet-}$ is an important oxidant in VLA NF-TiO_2 photocatalysis. The inhibition of the MC-LR photocatalytic degradation was more pronounced with the addition of SOD and catalase. SOD catalyzes the conversion of $O_2^{\bullet-}$ into H_2O_2 and oxygen, while catalase converts H_2O_2 to water and oxygen. H_2O_2 is produced by SOD, which can lead to the formation of •OH (see Section 3.1) in the absence of catalase although this could be a very low yield pathway. At pH 5.7, complete inhibition was observed with 1,4-benzoquinone (Fig. 2b). Higher inhibition with SOD and catalase was also observed at pH 5.7 compared to pH 3.0. The pKa of 1,4-benzoquinone is 9.9 and 11.4 [29] so at both pH values the interaction with NF-TiO_2 was low. The isoelectric point of SOD occurs at pH 5.0 [30] and thus in solution pH above 5.0, SOD has an overall negative charge, which may enhance interaction with the positively charged surface of NF-TiO_2 at pH 5.7. In addition, the pKa for superoxide is 4.6. Thus, the neutral form is dominant ($HO_2^{\bullet}$) at pH below 4.6, whereas the anionic form ($O_2^{\bullet-}$) is favored above pH 4.6.

The reduction potential of conduction band electrons in NF-TiO_2 is considered equivalent to that of undoped TiO_2 [13,31] and capable of reducing molecular oxygen at the surface of the catalyst to $O_2^{\bullet-}$ under visible light irradiation. The superoxide radical

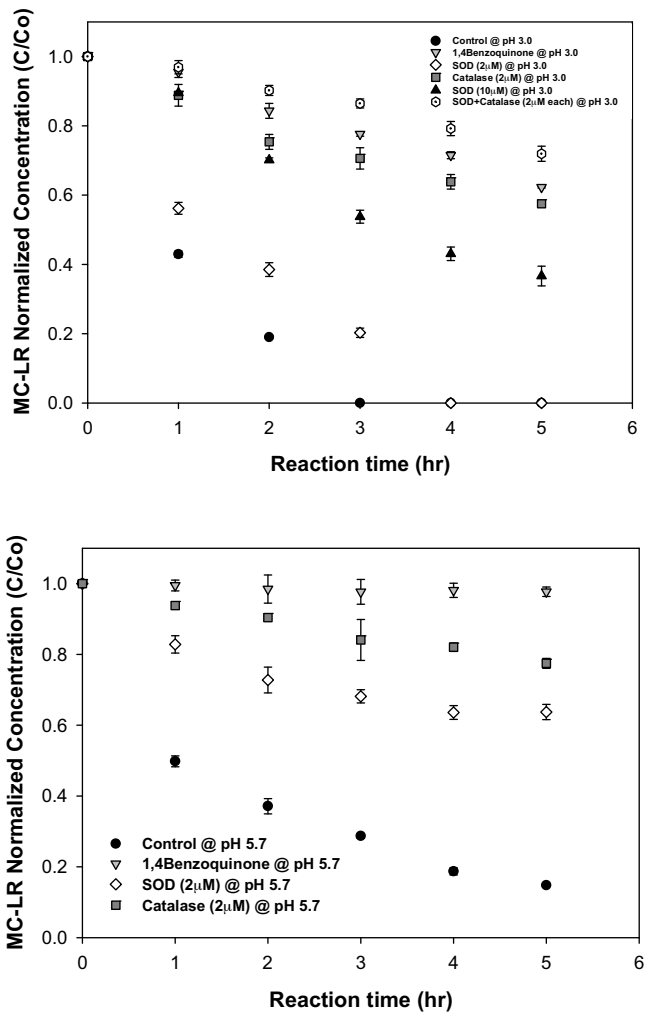


Fig. 2. Photocatalytic degradation of MC-LR with NF-TiO_2 in the absence and presence of SOD and 1,4-benzoquinone as $O_2^{\bullet-}$ scavenger and catalase as H_2O_2 scavenger under visible light at a) pH 3.0 and b) pH 5.7.

anion is a strong oxidizing agent with an electrochemical reduction potential of +0.89 V ($O_2^{\bullet-}/H_2O_2$) at pH 7.0 and can act as oxidizing species for the degradation of MC-LR [32]. The initial degradation of MC-LR was slower under visible light compared to UV or solar irradiation, which is consistent with the fact that VLA yields primarily $O_2^{\bullet-}$ which has a much less positive reduction potential than •OH (•OH/H_2O = +2.32 V at pH 7.0). Further reduction of $O_2^{\bullet-}$ with e^-_{CB} can produce H_2O_2 that could lead to •OH. The radical $O_2^{\bullet-}$ could also be further oxidized by holes to form singlet oxygen [13]. The production of $O_2^{\bullet-}$ has been detected using EPR during VLA N-TiO_2 photocatalysis [22]. According to recent EPR analysis [33], NF-TiO_2 comprises similar paramagnetic N-states to those of N-TiO_2 [22], with concentrations increasing dramatically upon visible light illumination, verifying the formation of localized intra-gap states above the valence band of TiO_2. These results indicate that the conduction band electrons excited from localized N-states by visible light in NF-TiO_2 can yield $O_2^{\bullet-}$ via electron transfer to surface adsorbed O_2.

3.3. *Prolonging $^1O_2$ lifetime*

To further explore the role of singlet oxygen in VLA NF-TiO_2 photocatalysis and eliminate the problems with competitive adsorption of scavengers, experiments were conducted with deuterium oxide (D_2O) as solvent. Singlet oxygen reactions are

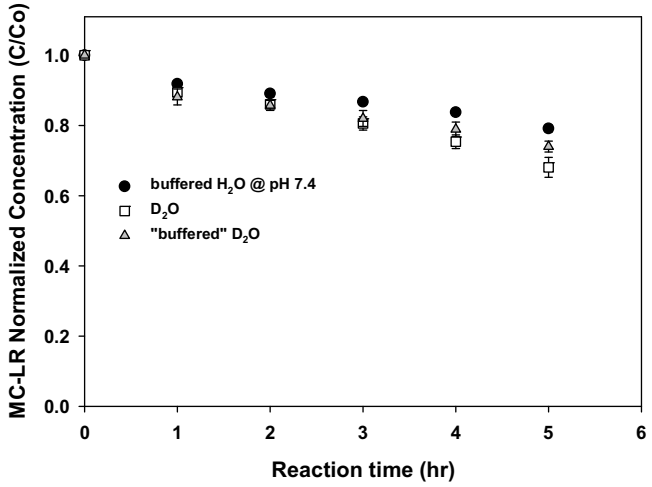


Fig. 3. Photocatalytic degradation of MC-LR with buffered H_2O and D_2O with NF-TiO_2 under visible light.

dramatically accelerated in D_2O compared to H_2O because the lifetime of singlet oxygen is 55 μs compared to 4.2 μs in H_2O [34]. The photocatalytic degradation of MC-LR was slightly higher in D_2O compared to H_2O buffered at pH 7.4 as illustrated in Fig. 3. Similar degradation of MC-LR was observed with buffered H_2O and buffered D_2O (pH 7.4) indicating minimal interaction of NF-TiO_2 with the ions from the buffered solution. At pH 7.4, electrostatic repulsion between negatively charged MC-LR and negatively charged NF-TiO_2 is expected to reduce the MC-LR adsorption but still ~30% degradation was observed. This indicates that reactive oxygen species are formed under D_2O as well but the results provide no direct indication on the presence of singlet oxygen. The formation of singlet oxygen is associated with the transformation of superoxide anion radicals. The redox potential of 0.34 V vs NHE indicates that the relation ($O_2^{\bullet-}/^1O_2$) is favored thermodynamically [13], but the formation of singlet oxygen is limited to the back electron transfer of an electron in $O_2^{\bullet-}$ to valence band holes. However, photogenerated holes are expected to remain localized in intra-gap energy states after charge separation in N-TiO_2 [22], which would limit the interaction with $O_2^{\bullet-}$ and the probability for the formation of singlet oxygen.

3.4. e^- and h^+ scavengers

In order to determine the involvement of e^-_{CB} and h^+ in NF-TiO_2 photocatalysis under visible light, cupric nitrate and formic acid were used as e^-_{CB} and h^+ scavengers, respectively. Rengaraj and Li employed formic acid as a hole scavenger to enhance the photocatalytic reduction reaction of Bi^{3+}-doped TiO_2 under UV light [35]. It can also inhibit the process by reacting with hydroxyl radicals and by competing with MC-LR for adsorption at the surface sites where ROS are produced. The inhibition of MC-LR in the presence of each scavenger is shown in Fig. 4a and 4b and compared to the control at pH 3.0 and 5.7. Under both pH values, partial inhibition of MC-LR degradation was observed. For Cu^{2+}, the adsorption of the transition metal onto NF-TiO_2 decreased the reduction of oxygen by conduction electrons and partially inhibited the formation of ROS and the removal of MC-LR. Chen et al., observed a hindering effect of Cu^{2+} ions on the photodegradation of three dyes using TiO_2 dispersions under visible light irradiation. The formation of ROS was blocked, based on EPR analysis, indicating that the adsorbed ions can alter the electron-transfer pathway and suppress the degradation of the dye [36]. Moreover, it is known that Cu^{2+} can be reduced by superoxide radical anion [37]. The competition for superoxide

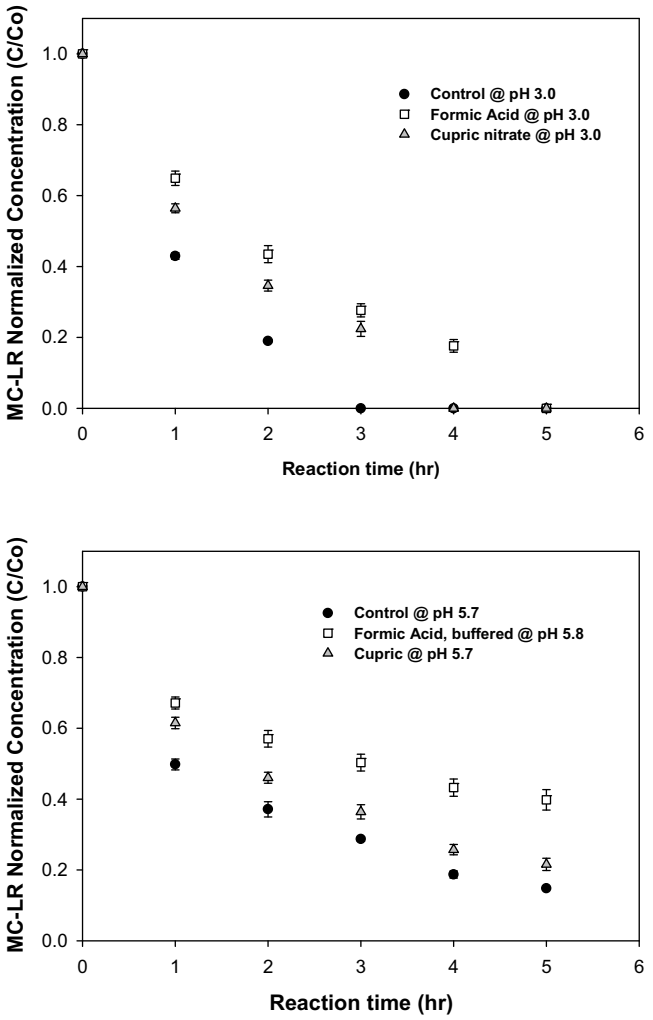
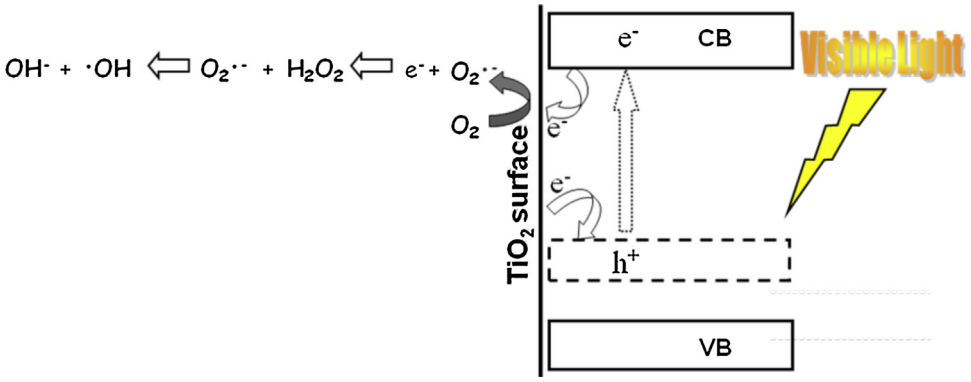


Fig. 4. Effect of $Cu(NO_3)_2$ and formic acid addition as e^- and h^+ scavenger, respectively, in NF-TiO_2 photocatalysis of MC-LR under visible light at a) pH 3.0 and b) pH 5.7.

radical anions between Cu^{2+} and MC-LR can lead to a reduction in the oxidation of the cyanotoxin. The addition of formic acid at pH 3.0 and buffered pH 5.7 is anticipated to prolong the lifetime of electrons and consequently produce more $O_2^{\bullet-}$, however inhibition of the photo-oxidation process was observed. VLA NF-TiO_2 photocatalysis does not generate a valence band hole that can lead to the formation of $^{\bullet}OH$. Therefore, the partial inhibition may result from the competition for adsorption between MC-LR and formic acid (pKa of 3.7) since formic acid can also interact with $^{\bullet}OH$ with rate constants k_{HA} of 1.3×10^8 M^{-1} s^{-1} and k_{A^-} of 3.2×10^9 M^{-1} s^{-1} [38] but high degradation of MC-LR was still observed indicating a lack of interaction with any potentially available $^{\bullet}OH$.

Scheme 1 shows the proposed mechanism of NF-TiO_2 photoactivation under visible light. Based on the findings obtained in this investigation, the mechanism of ROS formation for the synthesized photocatalyst under visible light is inferred to proceed via the reduction of molecular oxygen by visible light-excited electrons leading to the generation of superoxide radical anions and the intermediates formed and not via the generation of hydroxyl radicals by h^+ in the localized N-centers of NF-TiO_2. Superoxide is critical to the degradation process through disproportionation to H_2O_2 and the formation of $HO^{\bullet}$. The production of $HO^{\bullet}$ under VLA is relatively slow and in low yield, but localized at the sur-



Scheme 1. Proposed mechanism of NF-TiO_2 photoactivation under visible light.

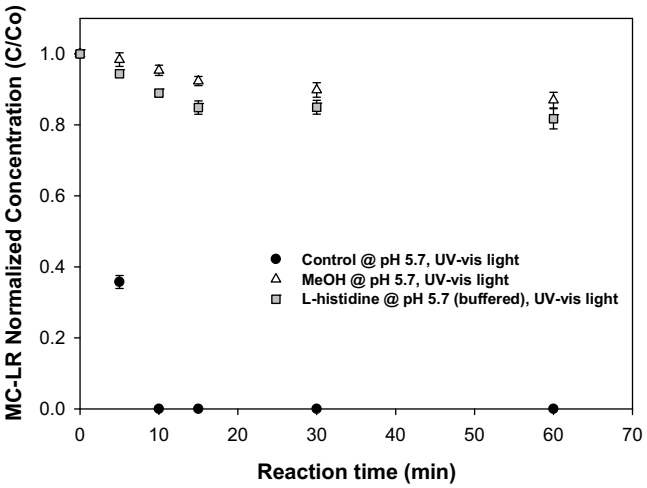


Fig. 5. Photocatalytic degradation of MC-LR with NF-TiO_2 under UV–vis irradiation and the scavenger effect of •OH with methanol and L-histidine.

face where adsorbates (such as MC-LR) are degraded. Adsorption strength plays a key role in the efficiency of the process.

3.5. •OH formation under UV–vis irradiation

Fig. 5 shows the photocatalytic degradation of MC-LR under UV–vis irradiation in the absence and presence of MeOH and L-histidine. Significantly faster removal of MC-LR was obtained with NF-TiO_2 under UV–vis irradiation as compared to visible light only irradiation at pH 5.7. In this case, the UV–vis source emitted UV radiation with relatively high intensity peaks at 310 and 365 nm [15]. The photoactivation of NF-TiO_2 with UV irradiation contributed to the enhanced performance of the photocatalyst towards the degradation of MC-LR. The possible generation of •OH under UV–vis irradiation with NF-TiO_2 could be responsible for this enhancement. In this case, high inhibition with MeOH and L-histidine was observed at pH 5.7. L-histidine is known to be a quencher for both $^1O_2$ and •OH with a pKa of 6.04 [39]. The second order rate constant of L-histidine with •OH is 5.0×10^9. Contrary to the results obtained under visible light, high inhibition of MC-LR was obtained in the presence of both scavengers. This indicates the generation of hydroxyl radicals as the primary reactive oxygen species with NF-TiO_2 under UV–vis irradiation. These results correlate well with our previous study using photoelectrochemistry to probe the mechanism in N,F-TiO_2 under UV and visible irradiation [40]. In that study, a small photocurrent under visible light excitation was observed, but there was an observable negative shift in the open circuit photopotential, which correlated well to the optical absorbance of the materials. The open circuit photopotential was less negative in the presence of oxygen, where some of the photoexcited electrons react with molecular oxygen to form superoxide radical anion and other ROS. There was a small increase in the visible light photocurrent in the presence of potassium superoxide, which provides some evidence for superoxide oxidation by inter-band gap holes to form singlet oxygen.

4. Conclusions

In this study, the mechanism of NF-TiO_2 radical formation was systematically investigated with selected scavengers for the degradation of MC-LR in water under visible light excitation. No direct indication for the presence of holes and •OH in NF-TiO_2 was obtained with formic acid and methanol, respectively, under visible light at pH 3.0 and 5.7. The formation of $O_2^{\bullet -}$ and H_2O_2 with NF-TiO_2 was established from the high inhibition of MC-LR degradation with superoxide dismutase and catalase at both pH values. Singlet oxygen formation is conditioned to further oxidation of $O_2^{\bullet -}$ with delocalized holes, whose formation could be limited in NF-TiO_2. When employing UV–vis irradiation, it was found that •OH is the primary ROS formed with NF-TiO_2 due to the high inhibition on the degradation of MC-LR with methanol and L-histidine, two •OH scavengers, at pH 5.7. Overall, pH plays a major role in the ROS formation during NF-TiO_2 photocatalysis under visible light for the degradation of MC-LR. The affinity of the scavenger with NF-TiO_2 and MC-LR as well as the disproportionation of the ROS needs consideration. Nevertheless, the results indicate that on NF-TiO_2 under visible light the ROS are generated via the oxygen reduction pathway driven by conduction band electrons, and the formation of hydroxyl radicals by valence band holes or holes in the mid-gap state does not play a significant role. This study provides essential information for understanding and improving the detailed reaction mechanism underlying the photocatalytic activity of NF-TiO_2 under visible and solar irradiation in the degradation of cyanotoxins and other contaminants of emerging concern in water.

Disclaimer

The research described in this article has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

Acknowledgments

This work was funded under the US-Ireland R&D Partnership by NSF and DELNI (NSF CBET-1033317) and the European Commission (Clean Water – Grant Agreement number 227017). Clean Water is a

Collaborative Project co-funded by the Research DG of the European Commission within the joint RTD activities of the Environment and NMP Thematic Priorities/FP7. P.F. acknowledges funding by Prince Sultan Bin Abdulaziz International Prize for Water (PSIPW)-Alternative Water Resources Prize 2014.

References

[1] M.R. Hoffmann, S.T. Martin, W. Choi, D.W. Bahnemann, Chem. Rev. 95 (1995) 69–96.
[2] S. Turchi, D.F. Ollis, J. Catal. 122 (1990) 178–192.
[3] S. Bhatkhande, V.G. Pangarkar, A. Beenacker, J. Chem. Technol. Biotechnol. 77 (2001) 102–116.
[4] A. Fujishima, T.N. Rao, D.A. Tryk, J. Photochem. Photobiol. C 1 (2000) 1–21.
[5] R. Asahi, T. Morikawa, K. Aoki, Y. Taga, Science 293 (2001) 269–271.
[6] S.G. Kumar, L.G. Devi, J. Phys. Chem. A 115 (46) (2011) 13211–13241.
[7] X.Z. Li, F.B. Li, Environ. Sci. Technol. 35 (2001) 2381–2387.
[8] N. Ghows, M.H. Entezari, Ultrason. Sonochem. 18 (2011) 629–634.
[9] M. Pelaez, N.T. Nolan, S.C. Pillai, M.K. Seery, P. Falaras, A.G. Kontos, P.S.M. Dunlop, J.W.J. Hamilton, J.A. Byrne, K. O'shea, M.H. Entezari, D.D. Dionysiou, Appl. Catal. B: Environ. 125 (2012) 331–349.
[10] C.E. Diaz-Uribe, M.C. Daza, F. Martínez, E.A. Páez-Mozo, C.L.B. Guedes, E. Di Mauro, J. Photochem. Photobiol. A 215 (2010) 172–178.
[11] S. Goldstein, D. Behar, J. Rabani, J. Phys. Chem. C 112 (2008) 15134–15139.
[12] H. Fu, L. Zhang, S. Zhang, Y. Zhu, J. Phys. Chem. B 110 (2006) 3061–3065.
[13] J.A. Rengifo-Herrera, K. Pierzchala, A. Sienkiewicz, L. Forro, J. Kiwi, C. Pulgarin, Appl. Catal. B 88 (2009) 398–406.
[14] I.-C. Kang, Q. Zhang, S. Yin, T. Sato, F. Saito, Environ. Sci. Technol. 42 (10) (2008) 3622–3626.
[15] S. Banerjee, S.C. Pillai, P. Falaras, K.E. O'shea, J.A. Byrne, D.D. Dionysiou, J. Phys. Chem. Lett. 5 (2014) 2543–2554.
[16] N.G. Moustakas, A.G. Kontos, V. Likodimos, F. Katsaros, N. Boukos, D. Tsoutsou, A. Dimoulas, G.E. Romanos, D.D. Dionysiou, P. Falaras, Appl. Catal. B 130–131 (2013) 14–24.
[17] M. Pelaez, A.A. de la Cruz, E. Stathatos, P. Falaras, D.D. Dionysiou, Catal. Today 144 (2009) 19–25.
[18] M. Pelaez, A.A. de la Cruz, K. O'shea, P. Falaras, D.D. Dionysiou, Water Res. 45 (2011) 3787–3796.
[19] A.A. de la Cruz, M.G. Antoniou, A. Hiskia, M. Pelaez, W. Song, K.E. O'Shea, X. He, D.D. Dionysiou, Anti-Cancer Agents Med. Chem. 11 (2011) 19–37.
[20] M.G. Antoniou, J.A. Shoemaker, A.A. de la Cruz, D.D. Dionysiou, Toxicon 51 (2008) 1103–1118.
[21] J. Wang, D.N. Tafen, J.P. Lewis, Z. Hong, A. Manivannan, M. Zhi, M. Li, N. Wu, J. Am. Chem. Soc. 131 (2009) 12290–12297.
[22] S. Livraghi, M.C. Paganini, E. Giamello, A. Selloni, C. Di Valentin, G. Pacchioni, J. Am. Chem. Soc. 128 (2006) 15666–15671.
[23] S. Kim, H. Park, W. Choi, J. Phys. Chem. B 108 (2004) 6402–6411.
[24] T. Hirakawa, Y. Nosaka, Langmuir 18 (2002) 3247–3254.
[25] M. Mrowetz, W. Balcerski, A.J. Colussi, M.R. Hoffmann, J. Phys. Chem. B 108 (45) (2004) 17269–17273.
[26] A.B. Murphy, Sol. Energy Mater. Sol. Cells 92 (2008) 363–367.
[27] N. Motohashi, Y. Saito, Chem. Pharm. Bull. 41 (1993) 1842–1845.
[28] W. Song, T. Xu, W.J. Cooper, D.D. Dionysiou, A.A. de la Cruz, K.E. O'Shea, Environ. Sci. Technol. 43 (5) (2009) 1487–1492.
[29] H. Nivinskas, S. Staskeviciene, J. Sarlauskas, R.L. Koder, A.F. Miller, N. Cenas, Arch. Biochem. Biophys. 403 (2002) 249–258.
[30] L. Banci, I. Bertini, P. Turano, Eur. Biophys. J. 19 (1991) 141–146.
[31] T. Hirakawa, Y. Nosaka, J. Phys. Chem. C 112 (2008) 15818–15823.
[32] P.M. Wood, Biochem. J. 253 (1988) 287–289.
[33] M. Pelaez, P. Falaras, V. Likodimos, A.G. Kontos, A.A. de la Cruz, K. O'shea, D.D. Dionysiou, Appl. Catal. B 99 (2010) 378–387.
[34] M.A.J. Rodgers, Activated oxygen, in: Ed Bensasson RV, Jori G, Land EJ, Truscott, Primary Photo-Processes in Biology and Medicine TG. NATO ASI Series A, Life Sciences (1984) 181–195.
[35] S. Rengaraj, X.Z. Li, Chemosphere 66 (5) (2007) 930–938.
[36] C. Chen, X. Li, W. Ma, J. Zhao, H. Hidaka, N. Serpone, J. Phys. Chem. B 106 (2) (2002) 318–324.
[37] Gianni Benzi, The modulating and toxic significance of endocellular peroxidations, in: Peroxidation, Energy Transduction and Mitochondria During Aging, John Libbey Eurotext, France, 1990, pp. 56.
[38] N.K.V. Leitner, M. Dore, J. Photochem. Photobiol. A 99 (1996) 137–143.
[39] A. Ahmed, P.-C. Yao, A.M. Brant, G.J. Peter, A.A. Harper, J. Biol. Chem. 272 (1) (1997) 125–130.
[40] J.A. Hamilton, J.A. Byrne, P.S.M. Dunlop, D.D. Dionysiou, M. Pelaez, K. O'Shea, D. Synnott, S.C. Pillai, J. Phys. Chem. C 118 (2014) 12206–12215.